EDITORIAL

Inorganic Nitrate: Marker or Mediator of Mortality?

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Nitrate is a relatively inert molecule generated in humans through the oxidation of endogenously synthesized nitric oxide (NO). We are also exposed to inorganic nitrate through dietary ingestion, with dietary exposure typically equaling or exceeding that of endogenous synthesis. There has been concern following work conducted in the 1970s that nitrate from the diet may be a precursor to the synthesis of carcinogenic *N*-nitrosamines in the human gastrointestinal tract.[1] This seemed to be supported by a number of epidemiological studies showing a correlation between nitrate exposure and a range of malignancies. The study by Maas et al in this issue of *JAHA* adds to this debate.

Since the discovery in 1994, by 2 independent groups, that inorganic nitrate may act as a source of nitric oxide (NO) in humans,[2,3] subsequent research has challenged the view that nitrate is harmful. It is clear that nitrate, whether from the diet or from the oxidation of endogenously synthesized NO, is involved in a complex enterosalivary circulation. Briefly, nitrate is concentrated in the salivary glands such that salivary nitrate concentrations are 10× that of plasma. Nitrate in saliva is reduced to nitrite by anaerobic bacteria on the tongue and swallowed. Some of this nitrite is converted to NO in the stomach, which appears to be important for regulation of gastric function and protection against enteric pathogens. Some nitrite is then absorbed into the circulation, such that plasma nitrite concentrations rise dramatically after a nitrate-rich meal (for example, green leafy vegetables or beetroot). Under conditions of hypoxia or acidosis, nitrite is further reduced to NO. This appears to provide an alternative source of NO when endogenous synthesis through the oxygen-dependent L-arginine NO synthase pathway is impaired. Trials of supplementation have suggested that adding nitrate to the diet can lower blood pressure, reduce the oxygen cost of exercise, and protect against ischemia–reperfusion injury,[4] although not all studies report such changes.[5]

The potential benefits of nitrate from the diet have led to calls from some quarters to raise the recommended daily intake. There do, however, remain a number of significant unanswered questions in the field.

Dietary nitrate supplementation studies have typically had small sample sizes and been of short duration.

There have been multiple large population studies suggesting harm related to nitrate ingestion.[6] Nitrate exposure is very difficult to estimate accurately, even beyond the well-known limitations of food diaries and food recall–based studies. Most of our exposure to nitrate comes from vegetables. The nitrate content of a given vegetable will vary according to where it grown, how much fertilizer is used, and even the time of day it is harvested.

Numerous studies have used red meat or processed meat as a proxy for nitrate intake. In 1 recent study, the National Institutes of Health–American Association of Retired Persons Diet and Health study, those in the highest category of meat-derived nitrate consumption were ingesting on average approximately 0.035 mmol/d from meat sources. Given that in the typical Western diet 1 to 2 mmol of nitrate is consumed, it seems unlikely that nitrate itself is a cause of the increased mortality associated with a meat-rich diet. When considering nitrate exposure from vegetables, evidence for a protective effect emerges.[7]

There are few population data with measured nitrate exposure to inform the debate one way or the other. Our group has recently shown an inverse association between higher 24-hour nitrate excretion and blood pressure in a model that included adjustment for antihypertensive medication; in this model, lower blood pressure would reasonably be expected to be associated with lower rates of cardiovascular disease.[8]

In this issue of *JAHA*, the examination by Maas et al of plasma nitrate and incidence of cardiovascular disease and all-cause mortality from the Framingham Offspring Study is the largest data set using a robust measure of plasma nitrate performed to date, with a median follow-up of 17.3 years.[9]

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In a model adjusted for age, sex, body mass index, systolic blood pressure, antihypertensive medication, smoking, diabetes mellitus, the total high-density lipoprotein cholesterol ratio, heart rate, and high alcohol consumption and C-reactive protein, they found a hazard ratio of 1.21 (1.04–1.40) for all-cause mortality for each 1-unit increase in ln-nitrate (P=0.015). When further adjusted for estimated glomerular filtration rate, this association was weakened (1.16 [1.00–1.35], P=0.057). There was no association with incident cardiovascular disease.

It is notable that in conditions known to have a causal relationship with mortality (impaired kidney function, smoking, hypertension, and diabetes mellitus), plasma nitrate was higher in this cohort. This is somewhat surprising in that, in at least some of these settings, NO synthesis has been demonstrated to be lower than in controls.[10,11] Why then, is plasma nitrate higher in these groups?

It has previously been suggested that circulating nitrite reflects endothelial NO synthesis, whereas nitrate concentrations do not.[12] The short duration of such experiments, the relative abundance of nitrate compared with nitrite in plasma, and difference in stability of these 2 products mean that this conclusion cannot be supported by data in the literature. In experiments of longer duration in animals, it is clear that in the absence of exogenous sources of nitrate, there is a substantial contribution to circulating nitrate concentrations from endothelial NO synthesis.[13] Based on these findings, plasma nitrate concentration can provide insights into vascular health in low dietary nitrate conditions.

The data from Kelm's group raise the possibility of a paradoxical relationship between plasma nitrate and nitrite concentrations and vascular health.[14] In healthy subjects, plasma nitrate concentration was 28±1 μmol/L and plasma nitrite concentration was 351±13 nmol/L. For those with 4 or more cardiovascular risk factors, the plasma nitrate concentration was 35±4 μmol/L and plasma nitrite concentration was 171±29 nmol/L (both P<0.001). This higher plasma nitrate concentration with increasing numbers of cardiovascular risk factors appears to be similar to the findings by Maas et al.[9] With increasing numbers of cardiovascular risk factors, plasma nitrite appears to be lower. Because of the approximately 100-fold difference in concentrations of these 2 anions, the differences in plasma concentrations between subjects with differing risk factors cannot be explained by conversion of one to the other. We have observed something similar in a cohort with diabetes mellitus compared with healthy controls.[15] Following placebo supplementation, there was a trend towards higher plasma nitrate in the subjects with diabetes mellitus, though this did not reach significance. Plasma nitrite concentration was lower in subjects with diabetes mellitus compared with controls: 232 (200, 265) nmol/L versus 285 (245, 348) nmol/L, P=0.019.

While the data in the Maas study are from fasting samples, they do not solely reflect endogenous synthesis of nitrate. Nitrate from the diet is still excreted by the kidneys up to 48 hours after ingestion.[16] From these data it is not possible to establish whether any associations are from diet or endogenous synthesis alone.

The modest inverse association of plasma nitrate concentration with estimated glomerular filtration rate may partially explain the observations of Maas and colleagues and go some way to explaining an association with plasma nitrate concentration and mortality found here.[9] The finding that plasma nitrate concentration is higher in those with renal impairment is not, however, universally accepted.[17,18] The effect of declining renal function would be expected to have an impact on plasma nitrate concentration, given that around 65% of circulating nitrate is excreted by the kidneys within 48 hours.[16] It is perhaps worth noting that the fractional reabsorption of nitrate in the kidney is approximately 95% with no evidence of a transport maximum.[19]

We have argued previously that a substance that is actively secreted in saliva and avidly reabsorbed by the kidneys is unlikely to be an unwanted toxin.[4]

The physiology of the nitrate–nitrite–nitric oxide cycle and its role in health and disease remains poorly understood. It is unlikely that we will ever be able to perform a large enough study of sufficient duration to elucidate the health effects of long-term dietary nitrate supplementation. To better understand the role of the nitrate–nitrite–nitric oxide system in both health and pathological states over the long term, we need prospectively designed studies where both plasma nitrate and nitrite are measured along with 24-hour urinary nitrate excretion. In the meantime a vegetable-rich diet, and by extension a nitrate-rich diet, remains something that is likely to be of benefit.

Disclosures

None.

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